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SYNTHESIS AND BIOLOGICAL ACTIVITY OF A SERIES OF PIPERAZINE-2,3-DIONE CONTAINING PENICILLINS AND 6α-FORMAMIDOPENICILLINS

I. DERIVATIVES SUBSTITUTED AT C(5) OR C(6) OF THE PIPERAZINE RING

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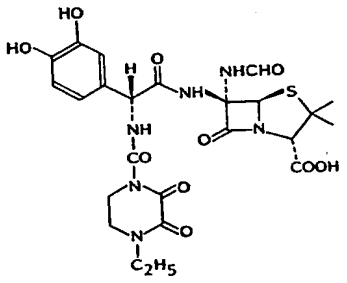
The synthesis and antibacterial activity of a series of penicillins and 6α-formamidopenicillins containing a C(5) or C(6)-substituted piperazine-2,3-dione moiety in the C(6)-β-sidechain is described.

The synthesis[1,2] and biological properties[3,4] of BRL 36650 (1), a C(6)-α-formamidopenicillin containing the 4-ethylpiperazine-2,3-dione group in the C(6)-β-sidechain, have been reported recently. BRL 36650 exhibits a potent level of activity against Gram-negative bacteria but, like temocillin (2)[5] and other C(6)-α-substituted penicillins[6,7], only poor activity against Gram-positive organisms. Replacement of the piperazine-2,3-dione moiety with a variety of different acyl radicals[7] failed to produce any new C(6)-α-formamidopenicillins with significantly enhanced activity against the Gram-positive bacteria. Piperacillin analogues (5a and 5b), having a substituent at the C(6)-position of the piperazine-2,3-dione ring, have previously been described in the literature[8] and 5a was shown to possess increased antibacterial activity and β-lactamase stability in comparison with piperacillin (3) itself. We decided to investigate the effect of substitution at C(5) and C(6) of the piperazine-2,3-dione residue in the hope of expanding the antibacterial spectrum of BRL 36650 (1) to incorporate activity against Gram-positive bacteria. Substitution in this manner generates an asymmetric centre in the piperazine-2,3-dione ring and it was apparent from the literature[8] that the stereochemistry of this centre had a pronounced effect on the potency of the subsequently derived penicillins. We, therefore, decided to synthesise all possible isomers for each substituent.

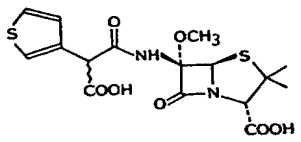
Chemistry

Piperazine-2,3-diones (4a~4d) were synthesised unambiguously from the appropriate *R* or *S*-amino or azido acids by known chemical methods[4,8]. However, piperazine-2,3-diones 4e and 4f were obtained as epimeric mixtures by subjecting the appropriate aldehydes to a modified Strecker procedure to give the aminonitrile[9], followed by reduction to the diamine[10] and cyclisation with diethyl oxalate[4].

As shown in Scheme 1, the carbonyl chlorides (8a~8f) were prepared by the action of phosgen [11], or, preferably, trichloromethyl chloroformate[12] on the silyl imino ethers derived from th piperazine-2,3-diones (4a~4f). Reaction of these carbonyl chlorides with ampicillin under standard Schotten-Baumann conditions[11] gave piperacillin analogues (5a~5d). Anhydrous acylation of the stable



1



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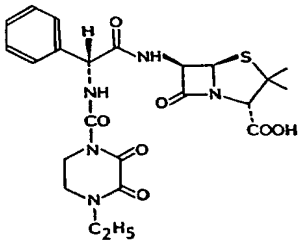

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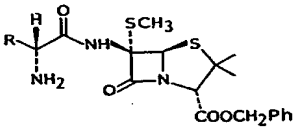
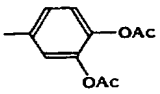
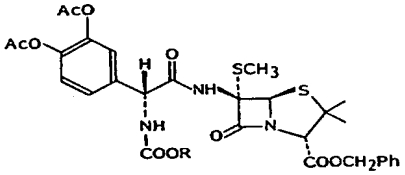
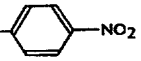


9a R=Ph

9b R= 



14a R= 

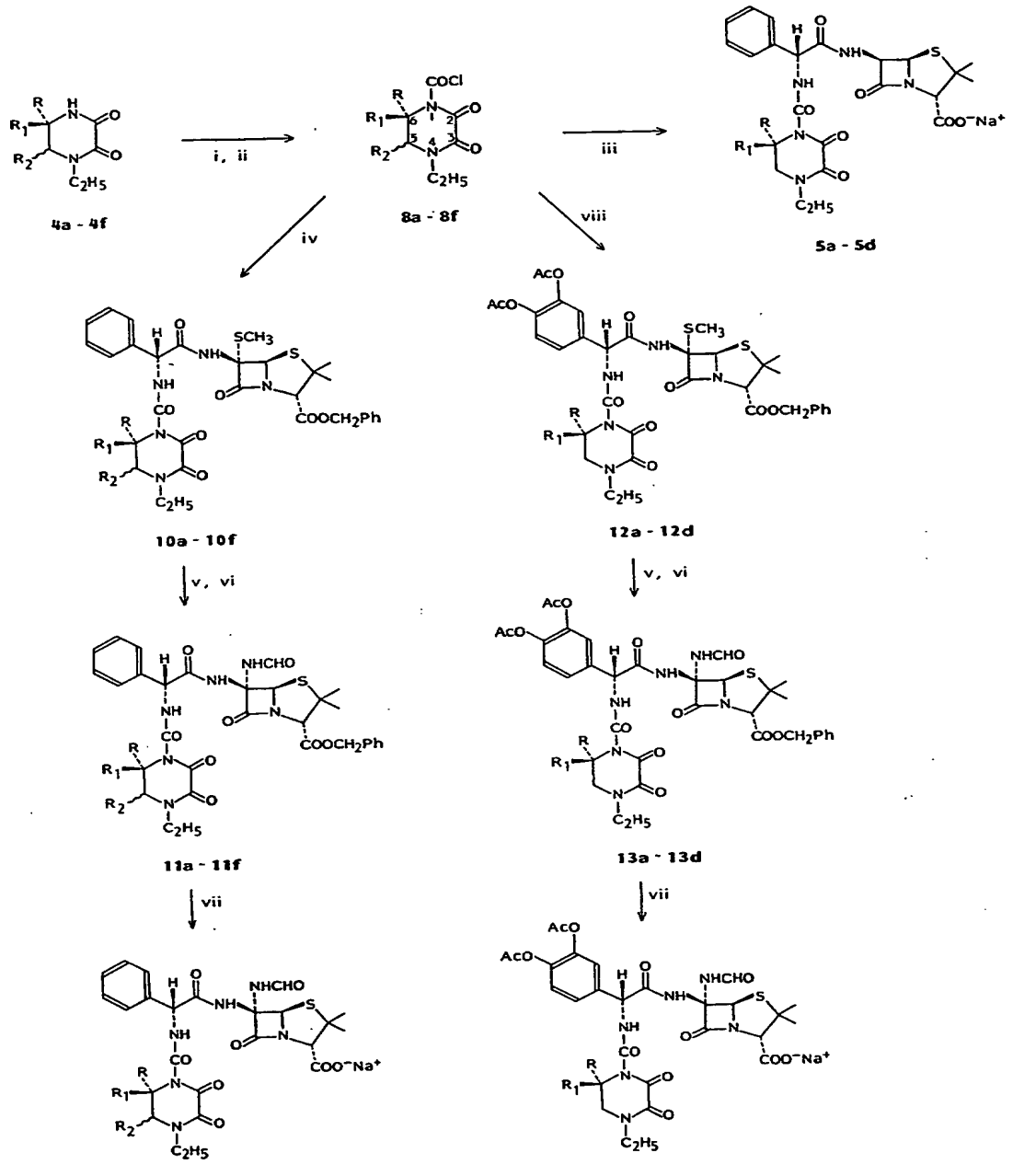
14b R=CH_2CCl_3

aminopenicillin (**9a**)[13] with carbonyl chlorides (**8a**~**8f**) gave the intermediate C(6)-α-methylthiopenicillins (**10a**~**10f**), which were converted to the C(6)-α-formamidopenicillins (**11a**~**11f**) by known methods[1]. Benzyl esters were then removed by catalytic hydrogenation to give the corresponding acids (**6a**~**6d**). The diastereoisomers of **11e** and **11f** were separated by silica gel chromatography and deprotected separately to give **6e***, **6e**$^+$, **6f*** and **6f**$^+$. The diacetoxypenicillins (**7a**~**7d**) were prepared by essentially the same process as the phenyl analogues. However, whereas **9a** is a stable, crystalline solid[13], the corresponding diacetoxy analogue (**9b**) is unstable to storage. The 4-nitrobenzyloxycarbonyl protected derivative (**14a**) was found to be stable and could be treated with zinc and hydrochloric acid to smoothly give the amine (**9b**). This was then acylated, without purification, to give esters (**12a**~**12d**). These intermediates were progressed *via* the C(6)-α-formamidopenicillins (**13a**~**13d**) to the corresponding acids (**7a**~**7d**) as described above. As we had previously found that the diacetoxy compounds were equipotent with the corresponding free catecholic derivatives, the acetate protecting groups were not removed. Although the trichloroethoxy carbamate (**14b**) is known in the literature and has been used to prepare **9b**[14], we found **14a** gave a more rapid and dependable preparation of the amine (**9b**).

Results and Discussion

The introduction of a C(6)-methyl or C(6)-phenyl substituent into the piperazine-2,3-dione ring of piperacillin (Table 1) gave compounds (**5a**~**5d**). In both series the *S*-isomers (**5a** and **5c**) were considerably more active than the *R*-isomers against Gram-negative bacteria. The C(6)-methyl analogue (**5a**) was marginally more active than the C(6)-phenyl compound (**5c**), but offered no advantage

Scheme 1.



a $R=CH_3$, $R_1=R_2=H$; b $R_1=CH_3$, $R=R_2=H$; c $R=Ph$, $R_1=R_2=H$; d $R_1=Ph$, $R=R_2=H$; e $R_2=CH_3$, $R=R_1=H$; f $R_2=Ph$, $R=R_1=H$; g $R=R_1=R_2=H$

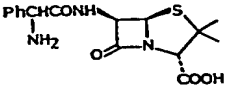
Reagents: i) $(CH_3)_3SiCl$, $N(C_2H_5)_3$; ii) $COCl_2$ or $ClCOOCCl_3$; iii)  ; iv) 9a, pyridine; v) $Hg(OAc)_2$, NH_3; vi) $CH_3COOCHO$, pyridine; vii) H_2 - Pd/C; viii) 9b, pyridine.

Table 1. *In vitro* activity of analogues 5 and 7 (MIC[a], μg/ml).

Organism	3	5a	5b	5c	5d	7a	7b	7c	7d	1
Escherichia coli DCO	1.0	1.0	4.0	2.0	64	0.25	2.0	0.5	16	≤0.03
E. coli DCO RTEM	64	128	>128	>128	>128	0.25	4.0	0.5	64	≤0.03
Klebsiella pneumoniae T767	8.0	4.0	32	4.0	128	0.25	4.0	0.5	>128	≤0.03
Enterobacter cloacae P99[b]	64	64	>128	128	>128	1.0	16	4.0	>128	0.5
Serratia marcescens US 32	0.5	2.0	16	2.0	64	1.0	8.0	4.0	>128	0.5
S. marcescens HCN 3956[b]	64	64	>128	128	>128	4.0	64	4.0	>128	1.0
Proteus mirabilis C977	0.5	1.0	16	2.0	32	2.0	32	16	>128	0.5
Pseudomonas aeruginosa 10662	4.0	4.0	16	8.0	64	2.0	16	4.0	64	0.12
Streptococcus pyogenes CN10	0.12	≤0.06	≤0.06	≤0.06	≤0.06	8.0	16	8.0	2.0	2.0
Staphylococcus aureus Oxford	1.0	0.5	0.5	0.5	0.5	>128	>128	128	>128	>128

[a] Serial dilution in Diagnostic Sensitest agar containing 5% defibrinated horse blood inoculated with 0.001 ml of an overnight broth culture diluted 1/100 (approx 10^4 cfu/spot).

[b] Constitutive class I β-lactamase producing strain.

Table 2. *In vitro* activity of C(6)-α-formamidopiperacillin analogues 6 (MIC[a], μg/ml).

Organism	6g	6a	6b	6c	6d	6e*	6e+	6f*	6f+
Escherichia coli DCO	4.0	2.0	32	4.0	>128	2.0	16	4.0	>128
E. coli DCO RTEM	4.0	2.0	128	4.0	>128	4.0	16	8.0	>128
Klebsiella pneumoniae T767	4.0	4.0	64	16	>128	4.0	32	8.0	>128
Enterobacter cloacae P99[b]	8.0	4.0	64	16	>128	8.0	16	16	>128
Serratia marcescens US 32	2.0	1.0	32	4.0	>128	2.0	8.0	8.0	>128
S. marcescens HCN 3956[b]	16	4.0	>128	4.0	>128	4.0	32	16	>128
Proteus mirabilis C977	2.0	2.0	32	16	>128	2.0	8.0	8.0	128
Pseudomonas aeruginosa 10662	16	32	>128	32	>128	16	>128	64	>128
Streptococcus pyogenes CN10	2.0	2.0	2.0	1.0	1.0	2.0	2.0	1.0	1.0
Staphylococcus aureus Oxford	>128	128	>128	128	128	>64	64	64	64

[a,b] See footnotes in Table 1.

over piperacillin itself against Gram-negative bacteria, although both penicillins were slightly more active against Gram-positive cocci.

Introduction of a C(6)-α-formamido group into piperacillin gave **6g**, which possessed considerably enhanced activity against β-lactamase producing Gram-negative bacteria such as *Escherichia coli* DCO (RTEM) and *Enterobacter cloacae* P99 as a result of improved β-lactamase stability. However, activity against Gram-positive strains was compromised. In this series (Table 2), the introduction of a C(6)-(*S*)-methyl substituent into the piperazine-2,3-dione moiety (**6a**) only led to a slight improvement in activity against Gram-negative bacteria relative to **6g**[7]. This effect was not seen with the corresponding phenyl analogue (**6c**) and neither substituent significantly enhanced the Gram-positive potency. Again, the corresponding *R*-diastereoisomers (**6b** and **6d**) showed reduced activity. In common with previous observations, compounds having methyl and phenyl substituents at the C(5) position of the piperazine-2,3-dione ring (**6e***, **6e**$^+$, **6f*** and **6f**$^+$) showed a considerable difference in activity between the two diastereoisomers. The more active penicillins (**6e*** and **6f***) were assumed, by analogy with the literature[8] and our own results presented herein, to be the *S*-isomers. Neither compound offered any significant advantage over the unsubstituted compound (**6g**)[7].

The remarkable effect of the introduction of the catechol residue (*cf.* BRL 36650) on Gram-negative potency, particularly against strains of *E. coli* and *Pseudomonas aeruginosa*, are now well known[3,4]. Substitution at C(6) of the piperazine-2,3-dione residue of BRL 36650 (**7a**~**7d**), failed to give compounds with enhanced Gram-positive activity and also adversely affected the Gram-negative potency of the derivatives (Table 1).

Conclusion

The improvements in antibacterial activity as a result of the introduction of a C(6)-α-formamido group into the piperacillin nucleus, and of the subsequent incorporation of a catechol into the glycine residue, have been reported previously[3,4]. Our results show that substitution at C(5) or C(6) of the piperazine-2,3-dione substituent did not further enhance these levels of activity.

Experimental

IR spectra were recorded for dichloromethane solutions on a Perkin-Elmer 197 spectrophotometer or for KBr discs on Perkin-Elmer 457 or Perkin-Elmer 983 grating spectrophotometers. ^{1}H NMR spectra were obtained on Perkin-Elmer R32 (90 MHz) or Bruker WM 250 (250 MHz) instruments using TMS as internal standard. While two rotameric forms were observed in the ^{1}H NMR spectra of the C(6)-α-formamidopenicillins, only the major, Z rotamer is quoted. Fast atom bombardment mass spectra (FAB-MS) were recorded on a VG ZAB spectrometer and the matrix is quoted.

Organic solutions were routinely dried over anhydrous magnesium sulfate and solvents were removed by evaporation under reduced pressure below 30°C. Preparative chromatography of penicillin esters was performed on Silica gel 60 (<230 mesh ASTM) (Merck 7729) and of penicillanic acid sodium salts on Diaion HP-20SS resin and the eluant is stated.

Hydrogenation of benzyl ester protecting groups was carried out in redistilled, dry THF in the presence of 10% palladium on carbon at atmospheric temperature and pressure and the catalyst removed by filtration through Celite.

Compounds used for antibacterial testing were all essentially single substances, analysed by reverse phase HPLC on a Gilson HPLC system. A μBondapak C-18 column, eluted with methanol in 0.05 M ammonium acetate (pH 4.5) was used and compounds detected by UV absorption at 240 nm.

Penicillanic acids (**5a** and **5b**) are known in the literature[8] and the C(5)-phenyl analogues (**5c** and **5d**) were prepared by the same method.

Benzyl (5*R*,6*S*)-6-[(2*R*)-2-((6*S*)-2,3-Dioxo-4-ethyl-6-methylpiperazin-1-ylcarbonylamino)-2-phenylacetamido]-6-methylthiopenicillanate (**10a**)

Amine (**9a**) (458 mg, 1 mmol) in THF (10 ml) containing triethylamine (101 mg, 1 mmol) was treated with a solution of **8a**, prepared from **4a** (156 mg, 1 mmol)[8], in THF (10 ml) at room temperature. The reaction mixture was stirred at room temperature for 0.5 hour then diluted with EtOAc and water. The separated organic phase was washed successively with water and saturated brine, dried and evaporated. Chromatography, eluting with 70% EtOAc in cyclohexane, gave the title compound (286 mg, 43%): IR (KBr) cm^{-1} 1780, 1747, 1714, 1699; ^1H NMR (250 MHz, $(CD_3)_2CO$) δ 1.02 (3H, s), 1.20 (3H, s), 1.18 (3H, t, J=7 Hz), 1.27 (3H, d, J=7 Hz), 2.32 (3H, s), 3.34~3.48 (2H, m), 3.53~3.64 (1H, m), 4.13 (1H, dd, J=5 and 13 Hz), 4.39 (1H, s), 4.74~4.79 (1H, m), 5.17 and 5.25 (2H, ABq, J=13 Hz), 5.43 (1H, s), 5.73 (1H, d, J=8 Hz), 7.28~7.58 (10H, m), 8.74 (1H, s, exchange), 10.01 (1H, d, J=8 Hz, exchange); FAB-MS (positive ion, carbowax - $CHCl_3$) m/z 668 (M+H, $C_{32}H_{37}N_5O_7S_2$).

Intermediates (**10b~10f**) were similarly prepared. **10e** and **10f** were obtained as diastereomeric mixtures.

Benzyl (5*R*,6*R*)-6-[(2*R*)-2-((6*S*)-2,3-Dioxo-4-ethyl-6-methylpiperazin-1-ylcarbonylamino)-2-phenylacetamido]-6-formamidopenicillanate (**11a**)

The C(6)-α-methylthiopenicillin (**10a**) was converted to the title compound by known methods[1] and **11a** was obtained in 46% yield after chromatography, eluting with EtOAc: IR (KBr) cm^{-1} 1785, 1742, 1690; ^1H NMR (250 MHz, $(CD_3)_2CO$) δ 0.92~1.29 (12H, m), 3.35~3.46 (2H, m), 3.53~3.64 (1H, m), 4.19 (1H, dd, J=6 and 13 Hz), 4.39 (1H, s), 4.75~4.77 (1H, m), 5.13~5.24 (2H, m), 5.57 (1H, s), 5.72 (1H, d, J=7 Hz), 7.28~7.60 (10H, m), 8.17 (1H, s), 8.25 (1H, br s, exchange), 8.80 (1H, br s, exchange), 10.06 (1H, d, J=7 Hz, exchange); FAB-MS (positive ion, 3-nitrobenzyl alcohol) m/z 665 (M+H, $C_{32}H_{36}N_6O_6S$).

Esters (**11b~11f**) were all prepared as described above. The diastereoisomers of **11e** and **11f** were separated by silica gel chromatography prior to deprotection.

Sodium (5*R*,6*R*)-6-[(2*R*)-2-((6*S*)-2,3-Dioxo-4-ethyl-6-methylpiperazin-1-ylcarbonylamino)-2-phenylacetamido]-6-formamidopenicillanate (**6a**)

Ester (**11a**) (115 mg, 0.17 mmol) was hydrogenated in THF (20 ml) and the crude product dissolved in dilute, aqueous sodium hydrogen carbonate solution and purified by chromatography, eluting with mixtures of THF in water (0~50% gradient). Concentration and freeze-drying of the relevant fractions gave **6a** (40 mg, 40%): IR (KBr) cm^{-1} 1772, 1684, 1611; ^1H NMR (250 MHz, $(CD_3)_2SO$) δ 0.82~1.18 (12H, m), 3.23~3.36 (2H, m), 3.40~3.59 (1H, m), 3.71 (1H, s), 3.80~3.97 (1H, m), 4.52~4.56 (1H, m), 5.37 (1H, s), 5.68 (1H, d, J=7 Hz), 7.22~7.54 (1H, m), 7.99 (1H, s), 9.16 (1H, br s, exchange), 9.80~10.00 (2H, m, exchange); FAB-MS (positive ion, thioglycerol) m/z 597 (M+H, $C_{23}H_{29}N_6O_6SNa$).

All penicillin sodium salts (**6b~6f**) were prepared as described above.

Benzyl (5*R*,6*S*)-6-[(2*R*)-2-(3,4-Diacetoxyphenyl)-2-((6*S*)-2,3-dioxo-4-ethyl-6-methylpiperazin-1-ylcarbonylamino)acetamido]-6-methylthiopenicillanate (**12a**)

The protected penicillin (**14a**) (780 mg, 1 mmol) was dissolved in THF (20 ml). Water (5 ml) then acid-washed zinc powder (1.56 g) were added and the pH of the mixture adjusted to 3.0 with 5 M HCl. The pH of the mixture was maintained between 3.0 and 5.0 by addition of more acid as required. When TLC showed little or no starting material remaining, the mixture was diluted with EtOAc and water and filtered through Celite. The phases were separated, the organic phase washed with saturated brine, dried and evaporated. The residue was dissolved in THF (10 ml) and treated sequentially with pyridine (79 mg, 1 mmol) then a solution of **8a**, prepared from **4a** (156 mg, 1 mmol)[8], in THF (10 ml). After 0.5 hour at room temperature, the reaction mixture was diluted with EtOAc and water and the phases separated. The organic phase was washed with water, saturated brine, dried and evaporated. Chromatography, eluting with 70% EtOAc in cyclohexane, gave **12a** (284 mg, 36%): IR ($CHCl_3$) cm^{-1} 1775, 1715, 1693; ^1H NMR (250 MHz, $(CD_3)_2CO$) δ 1.08 (3H, s), 1.18 (3H,

t, J=6 Hz), 1.25 (3H, s), 1.26 (3H, d, J=7 Hz), 2.24 (3H, s), 2.26 (3H, s), 2.31 (3H, s), 3.30~3.50 (2H, m), 3.53~3.68 (1H, m), 4.12 (1H, dd, J=5 and 13 Hz), 4.40 (1H, s), 4.71~4.82 (1H, m), 5.21 (2H, s), 5.42 (1H, s), 5.85 (1H, d, J=8 Hz), 7.20~7.55 (8H, m), 8.89 (1H, br s, exchange), 10.07 (1H, d, J=8 Hz, exchange); FAB-MS (positive ion, thioglycerol) m/z 784 (M+H, $C_{36}H_{41}N_5O_{11}S_2$).

Compounds (**12b~12d**) were similarly prepared and progressed through the formamido esters (**13a~13d**) to the acids (**7a~7d**) as described above.

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